

# The Effect of Endophytic Fungi in Drought Resistance of *Lolium Perenne* in Iran (Isfahan)

## Condition

Jajarmi Vahid<sup>1</sup>, Bihamta Mohamad R.<sup>2</sup>,  
Majidi Islam<sup>1</sup> and Darvish Farrokh<sup>1</sup>

<sup>1</sup>Department of Agriculture, Science and Research Branch, Islamic Azad  
University, Tehran, Iran

<sup>2</sup>Department of Agronomy and Plant Breeding, University of Tehran, Karaj, Iran

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### Abstract

It has been long that the symbiotic relationship among some members of poaceae family and endophyte fungi has been identified and under focus. They belong to genus *Epichloe* (Clavicipitaceae), which reside in the leaf sheath of *Lolium perenne*. The presence of endophytes in a plant leads to its resistance to a range of biotic and abiotic stresses. To conduct the study, more than 640 fresh plant materials (Natural samples) of *Lolium perenne*, of which 32 had endophytes, were collected from the native rangelands in north-east, west, north and northwest of Iran. To insure the presence of fungal endophyte in the samples, leaf sheaths were stained with Rose Bengal. The genomic DNA was also extracted from the isolates to confirm the identity of fungi. According to the morphological characteristics and specific primers, all the isolated fungi from *Lolium perenne* were identified as *Epichloe* endophytes. Then 32 endophyte-infected genotypes and 32 endophyte-free genotypes of *Lolium perenne* were planted in a farm in Isfahan. In order to study the role of endophytic fungi in drought resistance of *Lolium perenne*, an experiment was conducted in a randomized complete block design with two replications. To impose the drought stress, after determining the FC and PWP and

drawing the moisture diagram, when the soil moisture reached 40% of the field capacity, the field was irrigated with a volume of water that was calculated during the experiment appropriate with the root depth. In the present study, height, crown, dry weight, leaf length, leaf width, wet weight, number of tillers and leaf water content were measured. To analyze the data, SAS was employed and to compare the means LSD was used. The WARD was used to cluster-analyze the data and the Stat Graphic was used to draw the biplot. The stress conditions reduced all traits except leaf width and water content and this reduction was significantly lower in endophyte-infected genotypes. In other words, under the stress condition, endophyte-infected samples had higher tillers number, dry weight, crown, leaf length and wet weight. The cluster analysis separated the genotypes into 3 groups in the normal condition and four groups in the stress condition. The endophyte-infected genotypes and endophyte-free genotypes were accurately isolated. Most of the genotypes infected with endophytes were in the second group in normal condition. Enjoying most of the traits, the genotypes of this group had a better average than the other groups. The genotypes of this group are a very suitable source for breeding and performance increase. The isolation of the endophyte-infected and endophyte-free genotypes was properly carried out in the stress conditions of the cluster analysis and the endophyte-infected genotypes could go in the third group. The factor analysis also determined two factors that accounted for 88.92% of variation in the normal condition. In the genotype dispersion observed and evaluated in normal conditions, two groups were identified. The main factor included the number of tillers, wet weight. The coefficients in the second factor included the height that had a lower value in the first factor. Finally, under the stress condition, the factor analysis determined two factors that accounted for 86.15% of variation where the main factor included the number of tillers, dry and wet weight. The coefficients in the second factor included the crown and height. The genotype dispersion showed that most of the genotypes having endophytes were in the first quarter which, based on both the factors, stood at a very desirable level. One genotype that included endophytes could fit in the end part of the first quarter which, compared with the other genotypes, could stand at a better level. According cluster analysis and factor analysis, the trait average of the endophyte-free groups was lower than that of the other groups being in symbiotic relationship with endophytes. It can be concluded that the endophytes in normal and stress conditions can help increase the traits effective on performance.

**Keywords:** Endophytic Fungi, *Lolium perenne*, *Neotyphodium*, *Epcloe* endophytes Drought Stress, Factor Analysis, Cluster Analysis

## Introduction

Fungal endophytes have been found in many cool-season grass families of poaceae such as *Festuc*, *Lolium*, *Poa*, (Bacon, 1995), *Bromus* (White., 1987), *Hordeum*, *Stipa* (Wilson et al., 1991) and *Triticum* (Marshall et al., 1999). They

have been demonstrated to be from genus *Neotyphodium* consisting of *N.coenophialum* infecting tall fescue (*Festuca arundinacea*), *N.ancincinalum* infecting Meadow fescue (*Festuca pratensis*), and *N. loli* infecting perennial ryegrass (*Lolium preenne*) (White., 1987; Ren *et al.*,2007; Zhediannejad, Sabzalian and Mirlohi., 2013). Probably endophytes discovered in hordeum and triticum should be from genus *Epchloe* (Clavipitaceae). The results of the present study showed that the endophyte found in some of Iranian species like *Bromus tomentellus* belong to *Neotyphodium* (Mirlohi *et al.*, 2006). Interactions between *Epchloe* endophytes and infected plant of poaceae family produce many advantages such as tolerance to biotic stress like pest control, yield improvement (Latch *et al.*, 1985), tolerance to stressful environment like drought stress (Malinowski and Belesky, 2000) and mineral stress. Fungal endophytes produce a range of alkaloids such as lolin, peramin, ergowalin, and lolitrum that protect the host (plant) against insects (Malinowski and Belesky., 2000; Zhediannejad, Sabzalian and Mirlohi., 2013). According to Hatami, Mirlohi and Sabzalian (2006), endophyte-free plants were more susceptible to mealy bug, *p. solani* compared with endophyte-infected plants. This is due to alkaloid accumulation in rhizospher. Archavaleta *et al.*, (1989) showed the beneficial effects of endophytes in tall fescue and perennial ryegrass only at high Nitrogen concentrations. Under nutrient-limited conditions, endophyte decreases the metabolic cost on the host grass (Malinowski and Belesky., 2000). The results of the study "*The relationship of endophytic fungi with earliness and related traits in Tall Fescue (Festuca arundinacea) and Meadow Fescue (festuca pratensis)*" showed that traits such as days to head emergence, and days to maturity initiation reduced in the plant infected with endophytic fungi (Mirlohi, Sabzalian and Ehtemam., 2007). Fungal endophytes increased the length of root, number of tillers, head number, root weight, deeper crown (Parsaeiyan *et al.*, 2006; Sabzaliani *et al.*, 2004). Enhanced drought and salinity tolerance is documented in several studies of endophyte-infected species (West *et al.*, 1993;Sabzalin Mirlohi.,2010). The mechanisms involved are production of osmo-protectant compounds such as Manitol, Arabitol and Proline through endophyte-host interaction. Glucose and Fructose are produced in symbiotic plants (Sabzaliani *et al.*, 2004). Endophytic fungi stimulate secondary metabolites in response to drought tolerance. One of them, loline alkaloid, reduces the effect of drought stress (Bush *et al.*, 1997). Moreover, an increased photosynthesis in drought stress condition was reported (Marks and Clay, 1996). Stress is often described as an external factor that has a negative impact on a plant. Drought is the most common environmental (abiotic) stress which limits almost 25% of the production of the world. More than half of the area in Iran is grassland. The pastures provide food for livestock. Drought and water shortage have always been two of the most important agricultural and natural resources (grassland) problems in Iran, so that Iran has been classified as the world's arid region with an average precipitation of 240 mm in a year. High evapotranspiration rate, water resource restriction, and other factors have led to an increasing attention to the studies of drought effects and selection of drought-resistant cultivars (Genotypes). In Iran, some species of *Lolium* spread in the most

semi-arid, temperate zones and cold areas (Sharifan., 2008). Recent studies in Iran showed that *Lolium perenne* is adapted to both-cool-moist and cool-dry environments, and it grows in most areas, especially north and west of Iran. This great adaptability makes *Lolium perenne* valuable in soil conservation, rangeland recovery and forage production. *Lolium perenne* is native to the Iranian slopes. Endophytic fungi have also been found in *Lolium perenne* (Bacon., 1995; Sabzalian et al., 2004; Zhediannejad, Sabzalian and Mirlohi, 2013). Accordingly, the objectives of this study were

- a) to collect and evaluate the genetic diversity in *Lolium*
- b) *perenne* species of Iran, b) to investigate Iranian lolium coexisting with endophytic fungi,
- c) Morphological evaluation *Lolium perenne* in normal and drought conditions
- d) The effect of Endophytic fungi on *Lolium perenne*.

## Materials and Methods

### Identifying the *Neotyphodium* endophyte

Since an infected seed to endophytic fungi might be destroyed in the maintenance process, and since there was also a big selection of *lolium perenne* kinds, 640 natural samples (tender plants) were collected (during spring and summer, 2012, 2013) from the native rangelands in eastern north, north, west, western north and western regions of Iran (Fig.1).



Fig.1: *Lolium perenne* collection site map

In regard to the variety in each point, 10-20 natural samples were randomly and at a distance of 100-200 km were collected. To insure the presence of fungal endophyte in the samples, all leaf sheath samples were examined through using the SAHA technique (1988). To do so, leaf sheaths were soaked in sodium hydroxide (NaOH 5%, 2h). Then they were washed with distilled water, and finally stained with Rose Bengal, mounted and squashed under a cover slip and were finally investigated with a light microscope ( $\times 400$ ). To identify the *Neotyphodium* endophyte in the present study through the molecular way, it was

necessary to culture fungal endophytes to produce mycelia. For this purpose, leaf sheath parts (of half a centimeter length) were sterilized by being soaked in a 5% sodium hypochlorite for 10 minutes, and then the leaf sheath parts were washed in distilled water and being afterwards placed in flasks containing potato dextrose agave. Flasks were put in an incubator (22°C). To produce more mycelium, flasks were incubated on a platform shaker (100 rpm) at 25°C for 2 weeks. For DNA extraction, a modified CTAB protocol was followed (Zahediannejad, Sabzalian and Mirlohi, 2012; Mirlohi *et al.*, 2006). About 0.2 gr of mycelia was put in liquid nitrogen to produce a fine powder and then transferred to sterile centrifuge tubes containing 750 ml of extraction buffer, mixed well by inverting the tubes several times and incubated in a water bath at 60°C for 30 minutes. 750 ml of chloroform/isoamylalcohol (24:1) was then centrifuged at 10000 rpm for 15 minutes. The supernatant was poured off and DNA pellet was re-suspended in 100 sterile distilled water (Mirlohi *et al.*, 2006).

For PCR amplification of fungal DNA, two sets of specific primers including ITS1, ITS4 and IS1, IS3 were used. (Doss *et al.*, 1998).

name	sequence
ITS1	5'- GCGGCCATGTAGTAGAG-3'
ITS4	5'-TCCTCCGCTTATTGATATGC-3'
IS1	5'- GGTGTTGACCCCCCTGATTT-3'
IS3	5'- GTCTCATCTCCGGGGCGGTAT-3'

These primer sets amplified the fragmentation of 570 and 444 bp from *Neotyphodium* genomic DNA, respectively, although, to confirm the identity of *Neotyphodium* endophytes, other researchers had already applied three sets of specific primers (Mirlohi *et al.*, 2006). According to Mirlohi Protocol (2006), the reaction was performed in a 25 µL volume containing 2.5 µL of 1 × reaction buffer, 100 mM Tris-HCl pH 8.3, 15 mM MgCl<sub>2</sub>, 500 mM KCl, 2 mM each of dATP, dGTP, dCTP and dTTP, 12.5 µM of each primer, 0.75 U of *Taq* DNA polymerase and 100 ng of template DNA and then they were overlaid in 25 µl mineral oil. Amplification was performed in a DNA thermo-cycler that was programmed for one initial step cycle of denaturation at 95°C followed by 35 cycles consisting of denaturation for 1 minute at 94°C, primer annealing (1 minute at 60°C for IS1–IS3) and extension at 72°C for 2 minute. A final extension for 5 minutes at 72°C was followed for all primers. Samples were electrophoresed in a 1.2% agarose gel by using 1 × TBE buffer (Sambrook *et al.*, 1989) and visualized under UV light (Mirlohi *et al.*, 2006).

### Drought stress

After identifying 32 endophyte-infected genotypes, 32 endophyte-free genotypes of *lolium perenne* were selected. Both groups were planted in a farm of Isfahan. In order to study the effect of drought stress (irrigation at 40% - FC) on morphological traits in *lolium perenne*, an experiment was conducted in a randomized complete block design with two replications in 2013. The geographi-

cal coordinates of Isfahan are 51° 39' 40" E, 32° 38' 30" N. In the present study, height, crown, dry weight, leaf length, leaf width, wet weight, number of tillers and water content were investigated. In the present experiment, first the soil texture was determined and after the field had been fully irrigated and after 24 hours, where the field had gotten half-dry and the FC had been determined, the PWP was calculated for the *Lolium perenne* with no irrigation. With the moisture diagram drawn and the soil moisture having reached 40% of the field capacity, the volume of irrigation was determined with the following formulae used. To insure the presence of drought stress, the total available water and water volume for irrigation were determined.

$$TAW = 1000(\theta_{FC} + \theta_{PW})Z_r$$

FC=Field Capacity    PW= point of withered    Z<sub>r</sub>=Deep of root

To determine the water volume for irrigation, the following formula was used

$$d_N = \frac{FC - PWP}{100} \times Bd \times D \times Bd = \text{soil bulk density}$$

For normal condition (no stress), the volume of irrigation was 1064 cc and for stress condition 640 cc.

To analyze the data, SAS was employed and to compare the means LSD was used. The WARD was used to cluster-analyze the data and the Stat Graphic was used to draw the biplot.

## Results and Discussions

### A) Identifying the *Neotyphodium* endophyte

After staining the leaf sheath by Rose Bengal, the serpentine hyaline hyphae of the endophytes were observed in the leaf sheaths and aileron layer. Hyphae were 2-4 μm wide, parallel in leaf sheath and unbranched similar to endophytes of other reported hosts (Marshall et al., 1999; Zahediannejad et al., 2013). Of total tender plant natural samples of *loliumperenne* collected from different regions of Iran, just 32 natural samples had endophytes. Endophyte mycelia appeared after 30-52 days of leaf blade culture on PDA

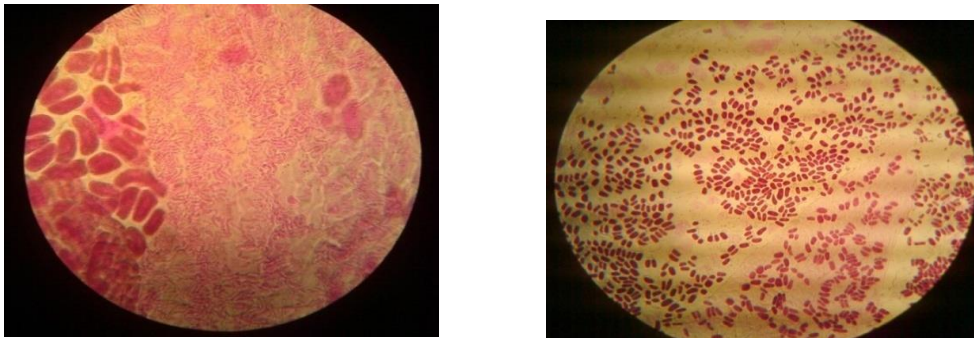


Fig. 2: Comparison of hyphae for endophyte-infected cells and endophyte-free cells

In similar studies, 30 Nano-grams DNA, 0.6 Microg/l for *Tag* DNA polymerase and 0.4 Microg/l for Mg Cl<sub>2</sub>, were the best measurements obtained for PCR (Zhedianneja, Sabzalian and Mirlohi., 2013) and therefore were employed in the present study. Accordingly, when primer IS1 and IS3 were tested, the intended 444-bp fragment was amplified from the DNA of the *lolium prene* isolates.

### B) Drought stress

The results showed that stress condition reduced the number of tillers, leaf length, height, leaf width and dry-wet weight. In a normal condition, the number of tillers was 198.09 but in a stress condition it was 166.02. When the effect of drought stress was being examined, the leaf width and water content were not suitable.

Table 1: Comparison of normal and stress conditions

Stress	Tiller	Leaf Length	Height	Crown	Leaf width	Dry weight	Wet weight	Water content
Normal	198.09 a	8.96 a	12.07 a	18.60 a	0.319 a	13.17 a	39.29 a	65.90 a
Stress	166.02 b	11.08 a	14.63 a	18.6 a	0.31 a	13.07 b	36.94 b	65.34 a

\*Means in the same column with different letters differ significantly at the 5% level of probability determined by using LSD test.

There was a significant difference between E+ and E- for all traits except % water and leaf width (Table 2). The number of tillers in endophyte-infected genotypes was 244.93, while in endophyte-free genotypes it was 51.18. The increased number of tillers in the stress condition is of importance. According to table 2, endophytes had a positive effect on the leaf length, height, crown, dry weight and wet weight. Endophytes increased the dry weight in *lolium perenne* from 9.89 to 16.34. Endophyte fungi have long been known to improve plant growth through increasing nutrient and water availability (Bacon, 1995; Hill *et al.*, 1990). Many studies have outlined the positive effect of endophytic fungi on plant growth and yield (Malinowski, Belesky.2000; Sabzalian *et al.*, 2006; Sabzalian, Mirlohi, 2010). Based on research, the mechanism involved is the production of osmoprotectant compounds such as Manitol, Arabitol and Priline through endophyte-host interaction (Mohamadi *et al.*, 2003). In these symbiotic plants, glucose is produced more significantly.

Table 2: Comparison of endophyte level and trait means

Endophyte	Tiller	Leaf Length	Height	Crown	Leaf width	Dry weight	Wet weight	Water content
E+	244.93 b	10.74 b	13.86 a	21.98 a	0.30 b	16.34 c	47.13 c	66.24 a
E -	151.18 d	9.57 c	12.84 b	14.96 d	0.32 a	9.89 d	29.10 d	65.03 a

\*Means in the same column with different letters differ significantly at the 5% level of probability determined by using LSD test.

### Cluster analysis

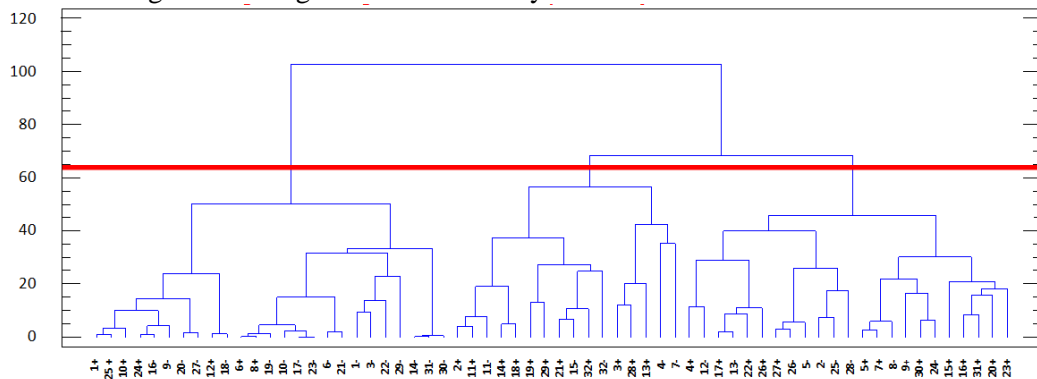
The dendrogram observed through the traits cluster analysis (WARD method) in the normal condition showed that genotypes were placed in three categories if

dendrogram cut off at a distance of 65. The first group had 25 members, and 60.39% of all genotypes belonged to it. The second group had 16 members, of which 9 genotypes were endophyte-infected and 7 genotypes were endophyte-free (Fig3). Endophyte-infected genotypes were mostly included in this group. The genotypes belonging to this group were a suitable source for the production of synthetic varieties and endophyte-free genotypes were placed in the third group. According to Hill (1990), environmental factors affecting the energy flow from the host to endophyte lead to a change in growth regulators and, as a result, increase the tiller number.

Table 3: Mean of traits in each group of cluster analysis in normal condition

Wet weight	Dry weight	Leaf width	Crown	Height	Leaf Length	Tiller	Cluster
23.13	11.15	0.12	13.14	9.47	9.17	64.54	<b>1</b>
352.28	141.84	0.34	39.04	25.43	18.16	556.00	<b>2</b>
165.42	65.72	0.23	31.88	14.86	14.43	317.65	<b>3</b>

Fig. 3: Dendrogram of cluster analysis in Isfahan normal condition

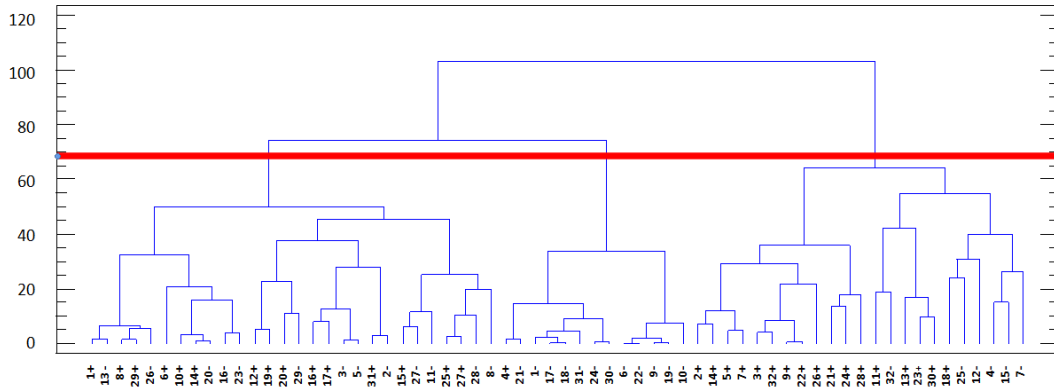


Cluster analysis for genotypes in the stress condition (WARD method) showed that genotypes were placed in three categories if the dendrogram were cut off at the distance of 68. The traits mean (Table 4) showed that the third group values, were higher than those of the groups without endophytes. In this group, the number of tillers was 313.24, the leaf length was 11.15 cm, the height 12.72 cm, and the crown 12.72 cm. The genotypes belonging to this group were a suitable source for the production of synthetic varieties and poly-cross.

Table 4: Mean of traits in each group of cluster analysis in stress condition

Wet weight	Dry weight	Leaf width	Crown	Height	Leaf Length	Tiller	Cluster
31.61	15.57	0.15	8.24	8.30	9.36	123.9	<b>1</b>
12.15	6.79	0.10	6.04	5.96	5.12	77.35	<b>2</b>
96.38	43.02	0.24	12.72	12.72	11.15	313.24	<b>3</b>

Fig. 4: Dendrogram of cluster analysis in Isfahan stress condition



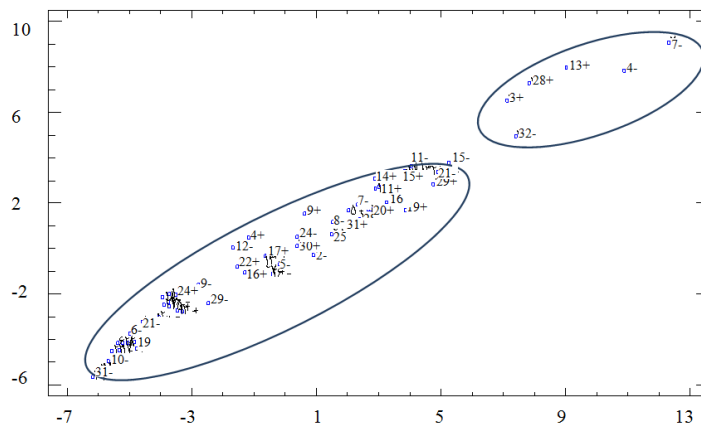
### Factor analysis

The factor analysis in the normal condition showed that 2 factors accounted for 88.92% of variation in the normal condition. The main factor included the number of tillers and dry and wet weight and the second factor included height (Table 5). In the genotype dispersion observed and evaluated in normal conditions, two groups were identified. A great majority of genotypes were in the group in the center of the diagram. The plants of this group included endophyte-infected and free genotypes.

Table 5: The trait coefficients in each effective factor of normal condition

First factor	Second factor	Traits
0.92	- 0.17	<b>Tiller</b>
0.87	0.25	<b>Leaf Length</b>
0.81	0.50	<b>Height</b>
0.93	0.08	<b>Crown</b>
0.90	- 0.29	<b>Leaf width</b>
0.93	- 0.93	<b>Dry weight</b>
0.96	- 0.96	<b>Wet weight</b>

Fig. 5: Biplot in a normal condition with 2 factors



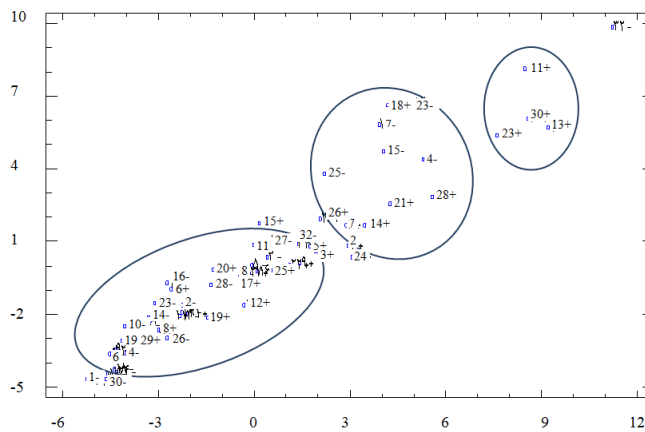
The group in the first quarter of the plot is inclusive of genotypes with and without endophytes where the genotypes of this area, in regard with their trait mean effective in the first and second factors, enjoyed higher coefficients. The factor analysis in the stress condition determined two factors that accounted for 85.62%. The first factor could gain 74.49 % of the variance of all traits, and the variables of tiller-number with a coefficient of 0.84, dry-weight 0.92, and wet weight 0.90 enjoyed higher coefficients (Table 6).

Table 6: The trait coefficients in each effective factor of stress condition

First factor	second factor	Traits
0.84	0.31	Tiller
0.50	0.57	Leaf Length
0.33	0.93	Height
0.33	0.93	Crown
0.73	0.55	Leaf width
0.92	0.31	Dry weight
0.90	0.37	Wet weight

The second factor could gain 11.65% of the variance of all traits where leaf-length with a coefficient of 0.50, leaf-width 0.73. The first group included the genotypes that were in the center of the plot and included the ones having a symbiotic and non-symbiotic relationship with endophytes that enjoyed average and low means of the effective traits in the two factors. The second group included the genotypes that were in the third quarter of the plot and these genotypes in symbiotic relationship with endophytes enjoyed trait with low means. The third group is in the beginning of the first quarter of the biplot table. A great many of the genotypes infected with endophytes were in this group which, in regard with their effective traits in the two factors, stood at a desirable level. There is also one genotype infected by endophytes in the end part of the first quarter which, compared with the genotypes infected by and free from endophytes enjoyed a trait average at a higher level.

Fig. 6: Biplot in the stress condition with 2 factors



## Conclusion

The Rose Bangal was a simple and suitable way for identifying endophyte fungi. *Lolium* reaction for all traits was not similar. Based on the traits among the endophyte-free loliums, the most appropriate genotypes were identified. The results indicated that endophytes had a positive effect on the number of tillers and the dry weight. The cluster analysis separated the populations of *lolium perenne* into three groups in the stress condition and it helped better distinguish the genotypes than the normal condition. The biplot separated genotypes in normal and stress conditions. Besides the genotypes including endophyte-infected ones, some endophyte-free genotypes were also identified. Breeding strategies should consider the presence or absence of endophytes in germplasm and the possible effect on variances.

## References

- [1] Arachevaleta, M., C. W. Bacon, C., S. Hoveland and D. E. Radcliffe. (1989). Effect of the tall fescue endophyte on plant response to environmental stress. *Agro. J.*81:83-90. <http://dx.doi.org/10.2134/agronj1989.00021962008100010015x>
- [2] Bacon, CW. and White, J. F. (1995). *Biotechnology of Endophytic Fungi of Grasses*. CRC Press, Boca Raton, Florida.
- [3] Bennett, J.S. (2000). Morphological differentiation in four species of the genus *lolium*. *Genetic Resources and Crop Evolution*. 47: 247-255. <http://dx.doi.org/10.1023/a:1008751026645>
- [4] Bush. L. P. and P.B. Jr. Barrus. (1988). Tall fescue forage quality and agronomic performance as affected by the endophyte. *Prod. Agric.* 1: 55-60. <http://dx.doi.org/10.2134/jpa1988.0055>
- [5] Doss, R.P. (1998). PCR-base technique for detection of *Neotyphodium* endophytes in diverse accessions of tall fescue. *Plant Dis.*82:738-740. <http://dx.doi.org/10.1094/pdis.1998.82.7.738>
- [6] Hill, T. L., C. P. West, K.E. Turner, M.E. Mc Connell and E. L. Piper. (1994). Incidence and viability of *Acremonium* endophytes in tall fescue and meadow fescue plant introductions. *Crop Sci.* 34:232-254. <http://dx.doi.org/10.2135/cropsci1994.0011183x003400010046x>
- [7] Malinowski, D. P. and D. P. Belesky. (2000). Adoptions of endophyte infect-cool season grasses to environmental stresses: Mechanism of drought and mineral stress tolerance. *Crop Sci.*40:923-940. <http://dx.doi.org/10.2135/cropsci2000.404923x>

- [8] Marks, S., and K. Clay. (1996). Physiological responses of *Festuca arundinaceae* to fungal endophyte infection. *New Phytol.*133:727-733.  
<http://dx.doi.org/10.1111/j.1469-8137.1996.tb01941.x>
- [9] Mirlohi, A., M. R. Sabzalian, B. Sharifnabi, and M.Khayam. (2006). Widespread occurrence of Neotyphodium-like endophyte in populations of *Bromustomentellus* in Iran. *Federation of European Microbiological Societies.FEMS.*256:126-131.  
<http://dx.doi.org/10.1111/j.1574-6968.2006.00101.x>
- [10] Parsaeiyan,M., Mirlohi,, AR.Rezaei and Bradaran. (2006). The morphologica changes affected by endophyte on induction of cold tolerance in two species of festuca. *Iranian journal of crop science.*vol.8No 2(30).
- [11] Marshall D, Tunal, B and Nelson LR. (1999). Occurrence of fungal endophytes in species of wild Triticum. *Crop Sic* 39: 1507-1512.  
<http://dx.doi.org/10.2135/cropsci1999.3951507x>
- [12] Sabzalian, M R., R Mohammadi and A.F.Mirlohi. (2004). Role of endophytic fungi in forage production of tall fescue, *Festucaarundinacea*. Forth international Iran and Russia conference.Shahr-e-kord.
- [13] Sabzalian ,M. R. and A. Mirlohi.(2010). Neotyphodium endophytes trigger salt resistance in tall and meadow fescues. *J. Plant Nutr. Soil Sci.* 173: 952–957.  
<http://dx.doi.org/10.1002/jpln.200900345>
- [14] Sabzalian, M. R., A. Mirlohi and B. Sharifnabi(2012). Reaction to powdery mildew fungus,Blumeria graminisin endophyte-infected and endophyte-free tall and meadow fescues. *Australasian Plant Pathol.* 41: 565–572.  
<http://dx.doi.org/10.1007/s13313-012-0147-1>
- [15] Sabzalian, M. R., B. Hatami and A. F.Mirlohi. (2004). Mealybug, Phenococcus solani, and barley aphid, Sipha maydis, response to endophyte-infected tall and meadow fescues.*Entomol. Exp. Appl.* 113: 205–209.  
<http://dx.doi.org/10.1111/j.0013-8703.2004.00227.x>
- [16] Sabzalian, M. R., R. Mohammadi, A. F Mirlohi and B. Hatami. (2004). Neotyphodium Fungi, Novel Bio-control Agents. The 15th international plant protection conference. China.
- [17] Sharifan,f et al.(2008).Genetic diversity in agronomic and morphological traits in lolium collection of national plant Gene Bank of Iran.10<sup>th</sup> Iranian congress of Crop Sci.18-20 Aug, 2008, karaj,IRAN.221-222.
- [18] White, J. F. (1987).Widespeed distribution of endophytes in Poaceae. *Plant*

Dis.71:340-342. <http://dx.doi.org/10.1094/pd-71-0340>

[19] Wilson, AD, clement SL and kaiserw (1991). Endophytic fungi in a *Hordeum* germplasm collection .FAO/ZBPGR.Plant Genetic Resources News letter 87:1-4.

[20] Viets, F. G. (1977). Effect drought control for successful dry land agriculture.

[21] Zahedian, M. Mirlohi and sabzalian.(2013).Detection of insecticidal alkaloids genes in *Neotyphodium* endophyt of five grass species, MS thesis. Isfahan university of technology. Isfahan, Iran.

**Received: May 4, 2015; Published: May 18, 2015**